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Synthesis and biological assays of E-ring analogs of camptothecin and homocamptothecin

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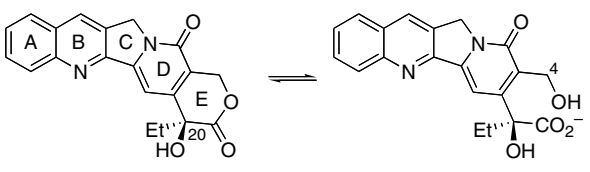
Dedicated to Professor Bernd Giese on the receipt of the 2005 Tetrahedron Prize.

Abstract—Analogs of the anti-tumor agent camptothecin with both closed E-rings (lactone and ether) and open E-rings (reduced acid, hydrazide, and protected Weinreb amide) have been prepared and tested in topoisomerase and cellular assays. The results provide insights into the structural features of the camptothecin E-ring that affect biological activity.

1. Introduction

Analogs of (*S*)-camptothecin **1** are among the most important current and prospective drugs for clinical treatment of solid tumors, and considerable information about structure/activity relationships in this family is now available.[1] The hydroxy-lactone E-ring of camptothecin is especially important from the standpoint of pharmacodynamics since equilibrium of the closed lactone form **1** with the open hydroxy-acid form **2** is relatively rapid under physiological conditions.[2]

It is often assumed that the open hydroxy acid form **2** of camptothecin is inactive; however, a recent crystal structure of a ternary complex of topoisomerase 1/DNA and the drug topotecan (a semi-synthetic analog of camptothecin) showed that the drug was present in both closed and open forms.[3] This result suggests that the open form of topotecan, and by implication other open analogs, might be biologically active.



1, (*S*)-camptothecin closed lactone form

2, lactone-opened (hydroxy acid) form

That the α-hydroxy lactone E-ring of camptothecin is not a prerequisite for activity has been shown by the discovery of the (*R*)-homocamptothecin **3** (β-hydroxy lactone) family of analogs,[4] some members of which are exceptionally potent (Fig. 1).[5] Homocamptothecin lactones open slowly and irreversibly under physiologically relevant conditions and their open hydroxy acid forms are inactive.[6] That result calls into question the conclusion that open α-hydroxy acid derivatives of standard camptothecins are active.

Biologically active, non-lactone E-ring analogs of camptothecin or homocamptothecin could not open up, and would be valuable as mechanistic probes and drug candidates. A few analogs, including ether **4**[7] and most notably cyclopentanone **5**,[8] show biological activity in cell or topoisomerase 1 (Top1) assays.

We report herein several new classes of closed and open E-ring analogs of camptothecin and homocamptothecin. Among these, only the open form hydrazide analogs are

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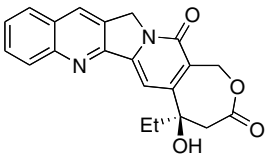
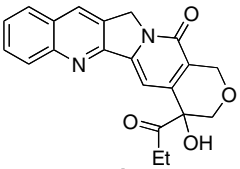


3, (*R*)-homocampothecin

non-lactone analogs



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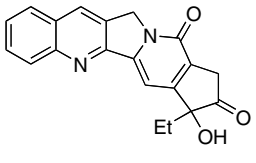

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Figure 1. Synthetic E-ring analogs of camptothecin.

active, and even these are likely to express their activity by reclosure to the lactone form of the corresponding camptothecin during storage or assay. The results help to further define the features of the crucial camptothecin E-ring.

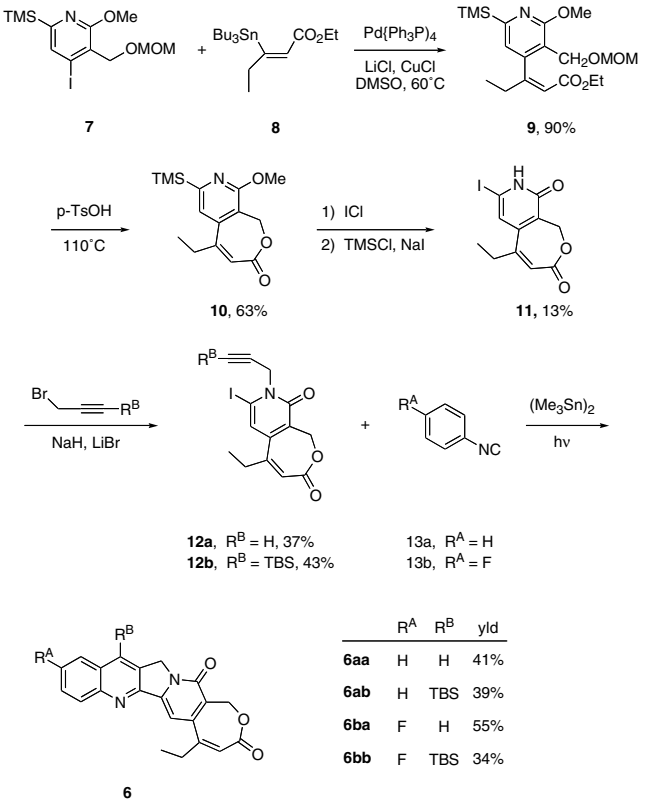
2. Results and discussion

2.1. Closed E-ring analogs

The first target for synthesis was achiral α,β-unsaturated homocamptothecin analog **6**. This is an interesting compound because it is the dehydration product of homocamptothecin **3**, and also because a related α-methylene lactone analog of camptothecin has been shown to be active.[9] However, bicyclic lactone **10** (see Scheme 1) proved difficult to prepare by a direct dehydration route.[10] This suggests that homocamptothecin does not readily dehydrate to **6** under physiological conditions.

Unsaturated lactone **6** was readily prepared by total synthesis as summarized in Scheme 1. Stille coupling of iodopyridine **7** with (*Z*)-vinylstannane **8** provided (*Z*)-α,β-unsaturated ester **9** in 90% yield.[11] Removal of the methoxymethyl (MOM) ether occurred with concomitant lactonization upon treatment of **9** with *p*-TsOH in refluxing toluene for 30 min to provide unsaturated lactone **10** in 63% yield. Lactone **10** is somewhat unstable under these acidic conditions and prolonged heating resulted in significantly lower yields.

Completion of the synthesis of **6** and several analogs followed the established steps of the cascade radical annulation route.[12] Low yielding iododesilylation of **10** with ICl (substantial quantities of starting material were recovered) followed by demethylation with TMSCl/NaI provided key iodolactone **11**. This was divided into two portions, which were *N*-alkylated with propargyl bromide and *tert*-butyldimethylsilyl (TBS) propargyl bromide to provide radical precursors **12a** and **12b** in 37% and 43% yields, respectively. Now cascade radical annulation of each of these two compounds with phenyl isonitrile **13a** and *p*-fluorophenyl isonitrile **13b** provided



	R^A	R^B	yld
6aa	H	H	41%
6ab	H	TBS	39%
6ba	F	H	55%
6bb	F	TBS	34%

Scheme 1. Synthesis of ene-lactone **6**.

unsaturated lactones **6aa** and three analogs **6ab**, **6ba**, and **6bb**. These analogs bear substituents that are expected to increase potency.

A number of the ene-lactones in this series of compounds exhibited interesting dynamic NMR behavior, and we studied lactone **10** in some detail to understand this behavior better. In the ^{1}H NMR spectrum of **10** at 300 MHz in $CDCl_3$, the diastereotopic methylene protons H4/H4′ exhibited a single resonance that was so broad as to be nearly invisible, while the remaining resonances were sharp and clear.[13] On warming to 50 °C, the resonance sharpened significantly, while cooling resulted in decoalescence, and two sharp doublets (J = 12.4 Hz) were observed at 5.51 and 4.78 ppm at −40 °C.[14]

We suggest that the dynamic process detected by these experiments is flipping of the seven-membered ring, as shown in Figure 2. A standard coalescence analysis[14] provides a barrier for this process of 13.3 kcal/mol for **10**, and we presume that other compounds, including **6**, have comparable barriers. In short, dehydration significantly alters both the shape and dynamics of the seven-membered lactone ring of homocamptothecin ene-lactone analogs **6**.

Access to the second closed analog, ether **17**, was facilitated by an accidental discovery. In an attempt to use **10** as an intermediate in the synthesis of homocamptothecin analogs, we treated it under standard epoxidation conditions with basic hydrogen peroxide. However, no epoxide was formed, and ring-contracted ether **14** was

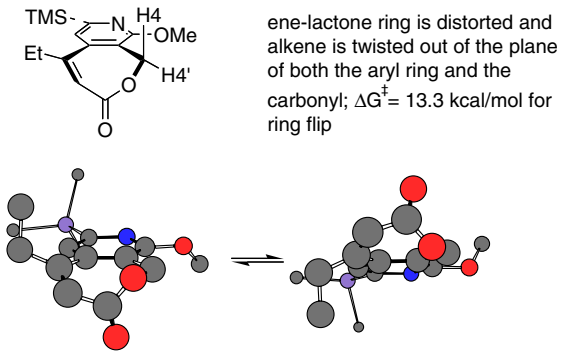
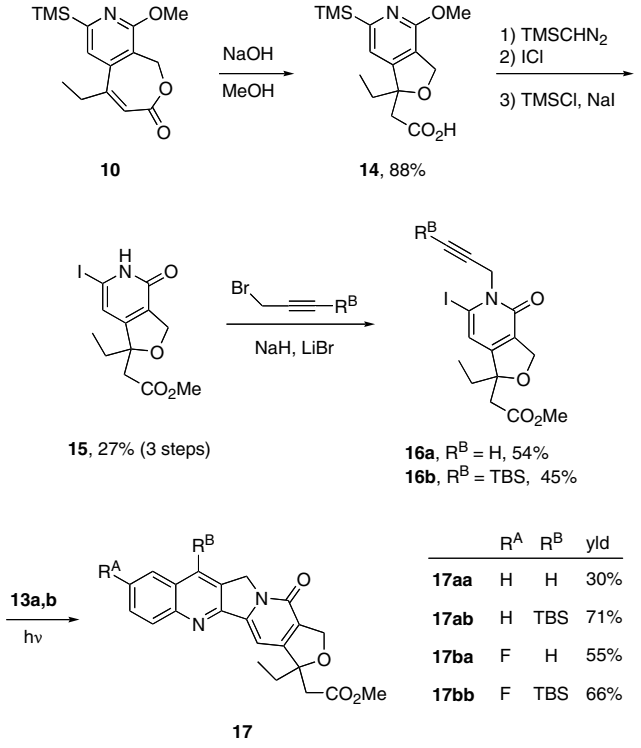


Figure 2. Proposed ring flip of unsaturated lactone **10**.



Scheme 2. Synthesis of ethers **17**.

isolated in 88% yield (Scheme 2). The hydrogen peroxide is not needed, and **10** rearranges cleanly to **14** on simple exposure to sodium hydroxide. The transformation presumably involved hydrolysis of the lactone to an α,β-unsaturated acid followed by conjugate addition of the liberated alcohol.

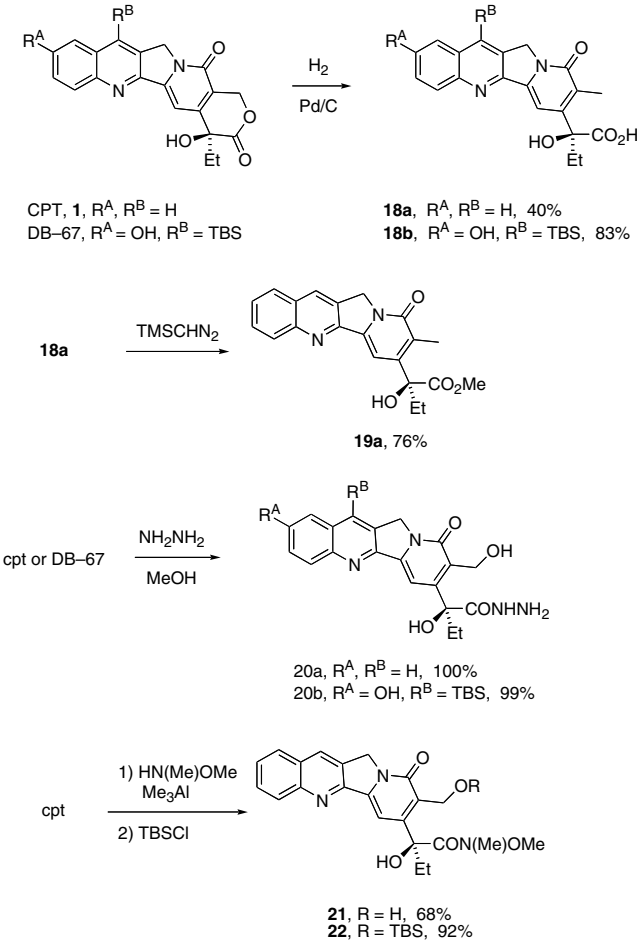
Acid **14** was esterified and the ester was taken through the same sequence of steps as in Scheme 1 to provide one iodolactone **15**, two propargyl lactones **16a** and **16b**, and finally four camptothecin analogs **17**. All of these analogs are racemates.

2.2. Open E-ring analogs

The suggestion that the open hydroxy acid form **2** of camptothecin might be biologically active is difficult to test experimentally because **2** and **1** can be expected to be in equilibrium under conditions of typical biological assays. It thus becomes of interest to synthesize open E-ring analogs of camptothecin that cannot or do not reclose under physiologically relevant conditions. We prepared several such potential analogs, as summarized in Scheme 3.

Hydrogenation of camptothecin and DB-67[15] provided hydroxyl acid analogs **18a** and **18b** in 40% and 83% yield, respectively. We suspect that the higher yield in the DB-67 series is due to the improved solubility in this series. Analogs **18a**,**b** are prohibited from reforming an E-ring under any conditions. Acid **18a** is a known compound[16] while **18b** is new. Both compounds can also be considered as analogs of the natural product mappicine.[17] Acid **18a** was also esterified to provide **19a**.

Reaction of camptothecin and DB-67 with hydrazine[18] provided acyl hydrazides **20a** and **20b** in excellent yields. These compounds were stable solids and were also stable toward chromatography and NMR analysis in solution, but they did not prove to have long-term solution stability (see below). We also prepared the Weinreb amide derivative **21** of camptothecin by a standard procedure;[19] however, this product was unstable on standing at room temperature according to TLC analysis. Conversion of **21** to the TBS ether **22** was accomplished as



Scheme 3. Synthesis of open E-ring analogs.

usual, and this compound proved to be stable to purification and storage.

2.3. Biological assays

All of the E-ring analogs were subjected to a standard topoisomerase 1 assay to test for activity.[6,20] Briefly, labeled DNA was incubated with recombinant Top1 with and without drug. After 20 min, the reaction was stopped and the samples were denatured and analyzed for DNA cleavage on a polyacrylamide gel.

All four of the cyclic ethers **17** were inactive in this assay at concentrations up to 100 μm. However, characteristic bands for DNA cleavage products were observed with the four unsaturated lactones **6**. The results with camptothecin analogs **6aa** and **6ba** are typical and are shown in Figure 3 in comparison to homocamptothecin **3**.[11] These analogs have modest activity and are roughly 100–1000 times less potent than the homocamptothecin standard samples. The two DB-67 analogs **6ab** and **6bb** exhibited comparable results (not shown).

The gel assay of several of the open E-ring analogs is shown in Figure 4. The reduced acids **18a** and **18b** are essentially inactive, as are the methyl ester **19a** and the protected Weinreb amide **21** (not shown). Interestingly, the acyl hydrazide samples **20a** and **20b** showed very high activity, essentially comparable to that of the positive control (camptothecin).

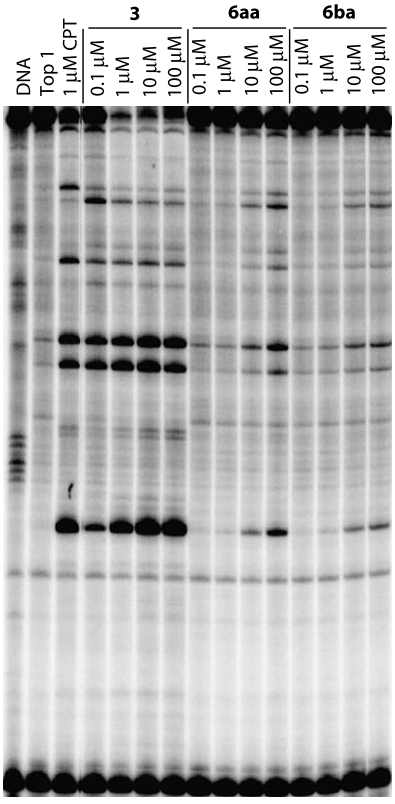


Figure 3. Gel electrophoresis of Top1-induced DNA cleavage assay for homocamptothecin **3** and E-ring lactone analogs **6aa** and **6ba**. Top1 is topoisomerase 1 without drug. Drug concentrations are indicated in each lane.

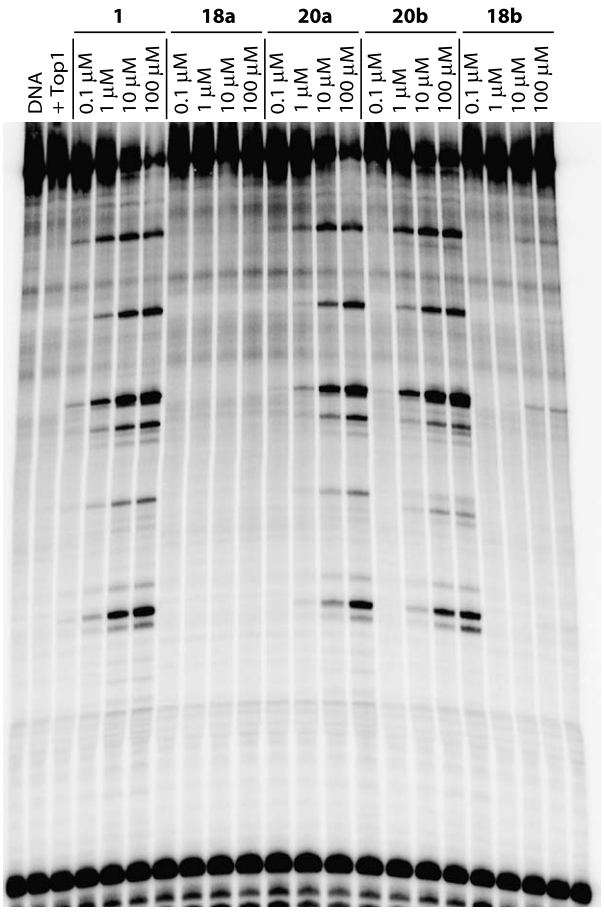


Figure 4. Gel electrophoresis of Top1-induced DNA cleavage assay for E-ring lactone analogs **18a**, **18b**, **20a**, and **20b**. Top1 is topoisomerase 1 without drug. Drug concentrations are indicated in each lane.

The open-chain analogs were also tested in a standard growth inhibition assay with MDA-MB-435S+ cells. Acids **18a** and **18b** were inactive up to >1 μm, but acyl hydrazide samples **20a** and **20b** were highly active, exhibiting GI_{50}'s of 20 and 100 nM, respectively. These values are comparable to the positive controls, camptothecin and DB-67 (~10 nM).

This high activity for **20a**,**b** led us to question whether these acyl hydrazides were stable under storage and assay conditions. Samples for assay were prepared and stored in DMSO, so we dissolved a small quantity of **20a** in DMSO-d_6 and allowed the sample to stand at ambient temperature. ^{1}H NMR spectra were periodically recorded, and we observed that after 20 days the hydrazide was completely absent and only camptothecin was present. A solid sample stored under the same conditions did not show any evidence of relactonization. Thus, the solid acyl hydrazides are stable, but the compounds are prone to relactonization in solution.

Several days after the topoisomerase assay, we also recovered one assay sample from the DMSO by extraction and found it to be a 2/1 mixture of camptothecin **1**

and acyl hydrazide **20a**. The composition of the sample under the actual assay conditions is not known (further conversion to the lactone form is possible), but it seems probable at this point that the activity exhibited by the acyl hydrazide samples **20a**,**b** is due predominantly or exclusively to the relactonization to the closed form under solution storage and/or assay conditions.

3. Conclusions

These results provide new insights into the role of the crucial E-lactone ring of camptothecin. Closed E-ring analogs ene-lactone **6** and ether **17** (non-lactone) are readily available by total synthesis through the cascade radical annulation route. While the ether series of compounds is inactive in topoisomerase assays, the unsaturated lactones exhibit modest activity. Being both homologs of camptothecin and lacking the key C20 hydroxyl group, these are some of the most distant E-ring analogs of camptothecin to retain activity. Yet the activity is greatly reduced, and in the big picture they are not that structurally distant. So the results reinforce the notion of the critical importance of the hydroxylactone ring.

Open acid analogs **18a**,**b** are very similar to **2**, the open hydroxy acid form of camptothecin (or DB-67), and differ only by the absence of the free hydroxy group at C4. It has been suggested that the open form **2** of camptothecin is active,[3] but the lack of activity of **18a** and **18b** does not support this suggestion. Since camptothecin itself does not have the C4 hydroxy group, it seems unlikely that this would be absolutely essential for binding of the open form **2** to the topoisomerase 1/DNA complex. So analogs **18** should have at least some activity in this assay if **2** is active, but they do not. Accordingly, our results support the conventional wisdom that open-chain hydroxy acid forms like **2** are not comparable to camptothecin in binding or biological activity.

The high activity of samples of acyl hydrazide coupled with their demonstrated propensity to relactonize in solution does not rigorously prove that these hydrazides are inactive. But it seems reasonable to conclude that most if not all of the activity exhibited by these samples in both topoisomerase and cell assays can be attributed to the presence of the analogous camptothecins formed by relactonization. Thus, these hydrazides, the Weinreb amides, and related molecules are potentially useful prodrugs for controlled targeting and release of camptothecin drugs.

4. Experimental

See Ref. 14 for general experimental details and copies of ^{1}H and ^{13}C NMR spectra of the E-ring analogs and key intermediates.

5-Ethyl-1-methoxy-3-(trimethylsilanyl)-9*H*-8-oxa-2-azabenzocyclohepten-7-one (10).[10] Stille coupling product **9**[11] was treated with *p*-TSA in refluxing toluene to provide **10** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (s, 9H), 1.16 (t, $J = 7.4$ Hz, 3H), 2.68 (q, $J = 7.4$ Hz, 2H), 4.01 (s, 3H), 6.37 (s, 1H), 7.11 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −2.2, 6.9, 19.9, 53.4, 60.4, 116.1, 118.4, 121.4, 144.9, 151.4, 160.2, 166.9, 167.18; IR (film, NaCl, cm^{-1}) 2964, 1723, 1550, 1451, 1345, 838; LRMS (70 eV, EI) *m*/*z* (rel int %) 291 (M^+), 276, 262 (100), 248, 232, 89, 73, 59; HRMS *m*/*z* calcd for $C_{15}H_{21}NO_3Si$ (M^+) 291.1291, found 191.1282.

5-Ethyl-3-iodo-1-methoxy-9*H*-8-oxa-2-azabenzocyclohepten-7-one. ICl (1 M in dichloromethane, 10 mL, 10 mmol) was added to a solution of **10** (0.73 g, 2.5 mmol) in CH_2Cl_2 (12 mL) kept at 0 °C in an ice bath and then allowed to warm to room temperature. After stirring for 16 h, the reaction mixture was poured into a chilled solution of 5% Na_2SO_3/brine (1:1, 150 mL) and extracted with ethyl acetate (3× 120 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (5:95 ethyl acetate/hexanes) to afford the iodide as a pale yellow oil (0.32 g, 38%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, $J = 7.3$ Hz, 3H), 2.62 (dq, $J = 1.3$ Hz, 7.4 Hz, 2H), 4.02 (s, 3H), 5.06 (br s, 2H), 6.38 (s, 1H), 7.38 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 28.9, 54.9, 60.2, 114.6, 116.4, 122.5, 124.4, 148.2, 149.4, 160.3, 167.3; IR (CH_2Cl_2, NaCl, cm^{-1}) 2955, 2924, 2847, 1736, 1618, 1454, 1362, 1045; HRMS (EI) *m*/*z* calcd for $C_{12}H_{12}INO_3$ (M^+) 344.9862, found 344.9868; LRMS (EI) *m*/*z* 345 (M^+, 100), 316 (58), 302 (74), 288 (54), 218 (22), 188 (35), 159 (43), 130 (55), 77 (36).

5-Ethyl-3-iodo-2,9-dihydro-8-oxa-2-azabenzocycloheptene-1,7-dione (11). Sodium iodide (0.15 g, 1.0 mmol) was added to a solution of the above iodide (0.12 g, 0.33 mmol) in dry acetonitrile (3.3 mL) at room temperature. Chlorotrimethylsilane (0.13 mL, 1.0 mmol) was then added and the reaction mixture was stirred for 15 min at room temperature. H_2O (3.0 μL, 0.17 mmol) was next added and the reaction mixture was heated to 60 °C and stirred at that temperature for 21 h. The mixture was then poured into a solution of 5% Na_2SO_3/brine (1:1, 20 mL) and quickly extracted with ethyl acetate (4× 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (1:4 acetone/dichloromethane) to afford **11** as a pale yellow solid (36 mg, 33%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (t, $J = 7.4$ Hz, 3H), 2.60 (q, $J = 7.2$ Hz, 2H), 4.99 (br s, 2H), 6.32 (s, 1H), 7.04 (s, 1H), 12.27 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 28.0, 29.0, 60.0, 122.2, 148.2, 149.3, 161.0, 166.7; IR (CH_2Cl_2, NaCl, cm^{-1}) 3344, 2955, 2837, 1710, 1629, 1583, 1444, 1014; HRMS (EI) *m*/*z* calcd for $C_{11}H_{10}INO_3$ (M^+) 330.9705, found 330.9717; LRMS (EI) *m*/*z* 331 (M^+, 100), 302 (82), 288 (92), 274 (65), 174 (17), 160 (26), 146 (18).

5-Ethyl-3-iodo-2-prop-2-ynyl-2,9-dihydro-8-oxa-2-azabenzocycloheptene-1,7-dione (12a). NaH in mineral oil (60%, 7.2 mg, 0.18 mmol) was added to a solution of **11** (30 mg, 0.091 mmol) in a mixture of DME (0.70 mL) and DMF (0.30 mL) at 0 °C under argon.

After stirring this mixture for 10 min at 0 °C, LiBr (32 mg, 0.36 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 15 min. Propargyl bromide (80% w/w in toluene, 80 μL, 0.72 mmol) was then added via a syringe and the reaction mixture was heated in the dark at 65 °C for 14 h. The final solution was poured into brine (10 mL) and extracted with ethyl acetate (3× 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (3:7 ethyl acetate/hexanes) to give **12a** as a pale yellow oil (12 mg, 37%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.3 Hz, 3H), 2.39 (t, J = 2.5 Hz, 1H), 2.55 (q, J = 7.4 Hz, 2H), 5.11 (s, 2H), 6.41 (s, 1H), 6.88 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 28.4, 29.7, 44.7, 61.1, 73.5, 101.1, 116.8, 123.8, 125.0, 148.0, 149.2, 159.8, 167.3; IR (CH_2Cl_2, NaCl, cm^{-1}) 2909, 2842, 2361, 2330, 1710, 1644, 1506, 1454, 1045; HRMS (EI) m/z calcd for $C_{14}H_{12}INO_3$ (M^+) 368.9862, found 368.9866; LRMS (EI) m/z 369 (M^+, 37), 340 (35), 326 (12), 256 (12), 149 (30), 129 (30), 73 (66), 57 (100).

2-[3-(*tert*-Butyl(dimethylsilanyl)prop-2-ynyl)]-5-ethyl-3-iodo-2,9-dihydro-8-oxa-2-azabenzocycloheptene-1,7-dione (12b). Following the above procedure, **11** (32 mg, 0.097 mmol) was alkylated with 3-*tert*-butyldimethylsilyl propargyl bromide (0.18 g, 0.77 mmol) in the presence of NaH in mineral oil (60%, 7.7 mg, 0.19 mmol) and LiBr (34 mg, 0.39 mmol) in a mixture of DME (0.75 mL) and DMF (0.31 mL). The crude product was purified by flash chromatography (1:4 ethyl acetate/hexanes) to afford **12b** as a colorless oil (20 mg, 43%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.91 (s, 9H), 1.17 (t, J = 7.4 Hz, 3H), 2.55 (q, J = 7.6 Hz, 2H), 5.13 (s, 2H), 6.41 (s, 1H), 6.88 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, 12.4, 16.6, 26.1, 28.4, 45.1, 61.3, 89.5, 98.5, 101.2, 116.7, 123.6, 124.9, 147.9, 149.4, 159.8, 167.4; IR (CH_2Cl_2, NaCl, cm^{-1}) 2955, 2847, 2248, 2176, 1726, 1649, 1511, 1265, 1035; HRMS (EI) m/z calcd for $C_{16}H_{17}INO_3Si$ ($M-{}^t$Bu) 426.0022, found 426.0023; LRMS (EI) m/z 426 ($M-{}^t$Bu, 25), 398 (19), 382 (22), 223 (10), 127 (56), 96 (100), 75 (91).

General procedure A: radical annulations. A solution of iodopyridone (~6–11 mg) in benzene was taken up in a 15 × 45 mm cylindrical screw-caped glass vial and kept at room temperature. A solution of isonitrile (1 M in benzene) and then hexamethylditin were added at room temperature. The vial was capped and the reaction mixture was irradiated with a 275 W GE sunlamp for 4 h. The solvent was then evaporated and the residue was purified by preparative thin-layer chromatography (1:9 acetone/dichloromethane).

5-Ethyl-1,13-dihydro-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (6aa). Following general procedure A, iodopyridone **12a** (7.2 mg, 0.020 mmol) was reacted with phenyl isonitrile **13a** (1 M, 78 μL, 0.078 mmol) and hexamethylditin (11 μL, 0.029 mmol) in benzene (0.33 mL) to afford **6aa**, after purification, as a yellow solid (2.0 mg, 41%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 2.81 (q, J = 7.3 Hz, 2H), 5.36 (s, 4H), 6.51 (t, J = 1.4 Hz, 1H), 7.49 (s, 1H), 7.71 (t, J = 7.4 Hz, 1H), 7.88 (t, J = 6.9 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 8.30 (d, J = 8.7 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 12.8, 29.3, 50.5, 61.0, 123.1, 125.8, 128.3, 128.4, 128.9, 130.9, 149.0, 151.3, 159.6, 167.8; IR (CH_2Cl_2, NaCl, cm^{-1}) 2914, 2847, 2361, 2335, 1710, 1644, 1598, 1444, 1035; HRMS (EI) m/z calcd for $C_{21}H_{16}N_2O_3$ (M^+) 344.1161, found 344.1161; LRMS (EI) m/z 344 (M^+, 55), 315 (70), 301 (45), 285 (35), 271 (20), 242 (32), 129 (17), 91 (46), 55 (100).

5-Ethyl-10-fluoro-1,13-dihydro-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (6ba). Following general procedure A, iodopyridone **12a** (7.8 mg, 0.021 mmol) was reacted with *p*-fluorophenyl isonitrile **13b** (1 M, 85 μL, 0.085 mmol) and hexamethylditin (12 μL, 0.032 mmol) in benzene (0.35 mL) to afford **6ba**, after purification, as a yellow solid (3.5 mg, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 2.80 (q, J = 7.3 Hz, 2H), 5.34 (s, 4H), 6.51 (s, 1H), 7.35 (s, 1H), 7.58 (dd, J = 2.5 Hz, 8.6 Hz, 1H), 7.61–7.65 (m, 1H), 8.24 (dd, J = 5.3 Hz, 9.2 Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.7, 29.1, 50.3, 60.8, 97.8, 111.3 (d, J_{CF} = 22.5 Hz), 121.2 (d, J_{CF} = 25.0 Hz), 123.1, 125.7, 129.0 (d, J_{CF} = 10.0 Hz), 129.6, 130.5, 132.3, 146.0, 146.3, 149.0, 151.1, 152.0, 159.5, 160.3, 162.3, 167.7; IR (CH_2Cl_2, NaCl, cm^{-1}) 2914, 2852, 2356, 2340, 1695, 1654, 1588, 1454, 1188, 1034; HRMS (EI) m/z calcd for $C_{21}H_{15}FN_2O_3$ (M^+) 362.1067, found 362.1068; LRMS (EI) m/z 362 (M^+, 22), 333 (30), 319 (19), 289 (7), 236 (7), 199 (12), 111 (25), 97 (46), 69 (75), 55 (100).

12-[*tert*-Butyl(dimethyl)silyl]-5-ethyl-1,13-dihydro-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (6ab). Following general procedure A, iodopyridone **12b** (11 mg, 0.023 mmol) was reacted with phenyl isonitrile **13a** (1 M, 91 μL, 0.091 mmol) and hexamethylditin (13 μL, 0.034 mmol) in benzene (0.38 mL) to afford **6ab**, after purification, as a yellow solid (4.0 mg, 39%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72 (s, 6H), 1.01 (s, 9H), 1.25 (t, J = 7.4 Hz, 3H), 2.81 (dq, J = 1.3 Hz, 7.4 Hz, 2H), 5.36 (s, 4H), 6.50 (t, J = 1.4 Hz, 1H), 7.37 (s, 1H), 7.64 (ddd, J = 1.5 Hz, 6.8 Hz, 8.4 Hz, 1H), 7.80 (ddd, J = 1.3 Hz, 6.8 Hz, 8.2 Hz, 1H), 8.24 (t, J = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −0.5, 12.7, 19.3, 27.2, 29.2, 52.8, 61.0, 97.5, 122.9, 125.1, 127.1, 129.6, 129.8, 130.7, 133.0, 136.3, 143.4, 146.7, 148.1, 149.0, 150.6, 151.4, 159.4, 167.9; IR (CH_2Cl_2, NaCl, cm^{-1}) 2914, 2842, 2351, 2335, 1726, 1649, 1598, 1465, 1045; HRMS (EI) m/z calcd for $C_{27}H_{30}N_2O_3Si$ (M^+) 458.2026, found 458.2028; LRMS (EI) m/z 458 (M^+, 100), 429 (58), 401 (47), 373 (63), 357 (46), 343 (16), 299 (7), 255 (7), 91 (8), 73 (15).

12-[*tert*-Butyl(dimethyl)silyl]-5-ethyl-10-fluoro-1,13-dihydro-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (6bb). Following general procedure A, iodopyridone **12b** (9.0 mg, 0.019 mmol) was reacted with *p*-fluorophenyl isonitrile **13b** (1 M, 75 μL, 0.075 mmol)

and hexamethylditin (11 μL, 0.028 mmol) in benzene (0.31 mL) to afford **6bb**, after purification, as a yellow solid (3.0 mg, 34%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72 (s, 6H), 1.01 (s, 9H), 1.25 (t, J = 7.4 Hz, 3H), 2.81 (dq, J = 1.3 Hz, 7.4 Hz, 2H), 5.35 (s, 4H), 6.50 (t, J = 1.4 Hz, 1H), 7.34 (s, 1H), 7.59 (ddd, J = 2.7 Hz, 7.5 Hz, 9.3 Hz, 1H), 7.89 (dd, J = 2.6 Hz, 11.0 Hz, 1H), 8.23 (dd, J = 6.0 Hz, 9.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.7, 12.7, 19.2, 27.1, 29.2, 52.8, 60.9, 97.4, 113.2 (d, J_{CF} = 23.8 Hz), 120.0 (d, J_{CF} = 25.0 Hz), 123.0, 125.2, 133.0 (d, J_{CF} = 102.5 Hz), 137.0, 142.5, 145.2, 146.4, 149.0, 150.3, 151.2, 159.3, 159.7 (d, J_{CF} = 26.2 Hz), 161.6, 167.8; IR (CH_2Cl_2, NaCl, cm^{-1}) 2955, 2914, 2842, 2361, 2330, 1716, 1659, 1593, 1214, 1040; HRMS (EI) *m/z* calcd for $C_{27}H_{29}FN_2O_3Si$ (M^+) 476.1932, found 476.1929; LRMS (EI) *m/z* 476 (M^+, 100), 447 (66), 433 (36), 419 (40), 391 (57), 375 (42), 361 (15), 273 (7), 98 (10), 73 (18).

(1-Ethyl-4-methoxy-6-trimethylsilanyl-1,3-dihydro-furo[3,4-*c*]pyridin-1-yl)acetic acid (14). H_2O_2 (30% w/w, 1.8 mL, 16 mmol) was added to a solution of **10** (0.30 g, 1.0 mmol) in MeOH (10.5 mL) kept at 0 °C in an ice bath. A solution of NaOH (6 N, 0.55 mL, 3.3 mmol) was added dropwise to this mixture via a syringe at 0 °C. After the addition was complete (5 min), the reaction mixture was warmed to room temperature and stirred for 6 h. Water (15 mL) was added, the layers were separated, and the aqueous layer was washed with CH_2Cl_2 (2× 15 mL). The aqueous layer was then acidified (pH ~ 3) by dropwise addition of 1 N HCl via a Pasteur pipet and subsequently washed with CH_2Cl_2 (2× 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford **14** as a colorless oil (0.28 g, 88%). The crude product was sufficiently pure for the subsequent reaction: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.29 (s, 9H), 0.77 (t, J = 7.3 Hz, 3H), 1.87 (dq, J = 14.7 Hz, 7.4 Hz, 1H), 2.01 (dq, J = 7.4 Hz, 14.6 Hz, 1H), 2.79 (d, J = 15.2 Hz, 1H), 2.81 (d, J = 15.2 Hz, 1H), 4.00 (s, 3H), 5.10 (d, J = 12.7 Hz, 1H), 5.12 (d, J = 12.8 Hz, 1H), 6.88 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 7.9, 32.2, 44.4, 52.9, 71.1, 89.6, 115.1, 120.1, 151.8, 158.2, 165.6, 173.9; IR (CH_2Cl_2, NaCl, cm^{-1}) 2950, 2858, 1705, 1582, 1449, 1352, 1239, 1029; HRMS (EI) *m/z* calcd for $C_{15}H_{23}NO_4Si$ (M^+) 309.1396, found 309.1382; LRMS (EI) *m/z* 309 (M^+, 17), 294 (27), 280 (62), 249 (65), 208 (20), 162 (9), 117 (10), 89 (37), 73 (100).

(1-Ethyl-4-methoxy-6-trimethylsilanyl-1,3-dihydro-furo[3,4-*c*]pyridin-1-yl)acetic acid methyl ester. $TMSCHN_2$ (2 M solution in hexanes, 0.55 mL, 1.09 mmol) was added to a solution of 14 (0.26 g, 0.84 mmol) in a mixture of methanol (1.5 mL) and benzene (5.3 mL) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was concentrated under reduced pressure to afford the ester as a yellow oil (0.26 g, 98%). The crude product was sufficiently pure for the subsequent reaction: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.29 (s, 9H), 0.76 (t, J = 7.3 Hz, 3H), 1.88 (dq, J = 7.4 Hz, 14.8 Hz, 1H), 2.01 (dq, J = 7.4 Hz, 14.7 Hz, 1H), 2.78 (d, J = 14.4 Hz, 1H), 2.80 (d, J = 14.4 Hz, 1H), 3.59 (s, 3H), 4.00 (s, 3H), 5.03 (d, J = 12.7 Hz, 1H), 5.05 (d, J = 12.7 Hz, 1H), 6.89 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 7.8, 32.5, 44.5, 51.4, 52.8, 70.9, 89.7, 115.5, 120.8, 152.4, 158.2, 165.0, 170.2; IR (CH_2Cl_2, NaCl, cm^{-1}) 2955, 2858, 1777, 1741, 1593, 1460, 1362, 1034; HRMS (EI) *m/z* calcd for $C_{16}H_{25}NO_4Si$ (M^+) 323.1553, found 323.1563; LRMS (EI) *m/z* 323 (M^+, 43), 308 (40), 294 (65), 250 (100), 234 (42), 208 (46), 84 (82).

(1-Ethyl-6-iodo-4-methoxy-1,3-dihydro-furo[3,4-*c*]pyridin-1-yl)acetic acid methyl ester. A solution of ICl (0.42 g, 2.6 mmol) in CCl_4 (1.82 mL) was added to a solution of the above ester (0.21 g, 0.65 mmol) in CH_2Cl_2 (2.6 mL) kept at 0 °C in an ice bath and allowed to warm to room temperature. After stirring for 14 h, the reaction mixture was poured into a chilled solution of 5% Na_2SO_3/brine (1:1, 40 mL) and extracted the mixture with ethyl acetate (3× 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (5:95 ethyl acetate/hexanes) to afford the iodide as a pale yellow oil (0.1 g, 41%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.60 (t, J = 7.2 Hz, 3H), 1.73 (m, 2H), 2.60 (d, J = 15.0 Hz, 1H), 2.62 (d, J = 15.0 Hz, 1H), 3.44 (s, 3H), 3.79 (s, 3H), 4.80 (d, J = 12.9 Hz, 1H), 4.82 (d, J = 12.9 Hz, 1H), 7.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.6, 32.6, 44.0, 51.5, 54.1, 70.5, 89.0, 111.3, 121.1, 121.4, 156.2, 157.5, 169.8; IR (CH_2Cl_2, NaCl, cm^{-1}) 2950, 2853, 2356, 2330, 1741, 1593, 1460, 1362, 1035, 850; HRMS (EI) *m/z* calcd for $C_{13}H_{16}INO_4$ (M^+) 377.0124, found 377.0129; LRMS (EI) *m/z* 377 (M^+, 32), 348 (35), 303 (100), 288 (23), 162 (28), 77 (20).

(1-Ethyl-6-iodo-4-oxo-1,3,4,5-tetrahydro-furo[3,4-*c*]pyridin-1-yl)acetic acid methyl ester (15). Sodium iodide (29 mg, 0.20 mmol) was added to a solution of the above iodide (46 mg, 0.12 mmol) in dry acetonitrile (1.2 mL) at room temperature. Chlorotrimethylsilane (25 μL, 0.20 mmol) was then added and the reaction mixture was stirred for 15 min at room temperature. H_2O (1.0 μL, 0.061 mmol) was added and the reaction mixture was heated at 60 °C for 22 h. The mixture was then poured into a solution of 5% Na_2SO_3/brine (1:1, 7.8 mL) and quickly extracted with ethyl acetate (4× 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (2:3 ethyl acetate/hexanes) to afford **15** as a pale yellow solid (30 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$, the amide NH was not located) δ 0.80 (t, J = 7.2 Hz, 3H), 1.86 (m, 2H), 2.75 (q, J = 14.8 Hz, 2H), 3.61 (s, 3H), 5.01 (s, 2H), 6.66 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.7, 32.2, 43.8, 51.7, 71.8, 90.2, 94.0, 113.2, 127.4, 155.8, 161.0, 169.8; IR (CH_2Cl_2, NaCl, cm^{-1}); HRMS (EI) *m/z* calcd for $C_{12}H_{14}INO_4$ (M^+) 362.9968, found 362.9961; LRMS (EI) *m/z* 363 (M^+, 22), 334 (31), 289 (100), 274 (15), 163 (23), 78 (21).

(1-Ethyl-6-iodo-4-oxo-5-prop-2-ynyl-1,3,4,5-tetrahydro-furo[3,4-*c*]pyridin-1-yl)acetic acid methyl ester (16a).

NaH in mineral oil (60%, 1.5 mg, 0.036 mmol) was added to a solution of **15** (12 mg, 0.033 mmol) in a mixture of DME (0.25 mL) and DMF (0.10 mL) at 0 °C under argon. After stirring this mixture for 10 min at 0 °C, LiBr (5.8 mg, 0.066 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 15 min. Propargyl bromide (80% w/w in toluene, 15 μL, 0.13 mmol) was then added via a syringe and the reaction mixture was heated in the dark at 65 °C for 7 h. The final solution was poured into brine (5 mL) and extracted with ethyl acetate (3× 5 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (2:3 ethyl acetate/hexanes) to give **16a** as a pale yellow foam (7.0 mg, 54%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, J = 7.4 Hz, 3H), 1.78 (dq, J = 7.4 Hz, 14.7 Hz, 1H), 1.92 (dq, J = 7.4 Hz, 14.7 Hz, 1H), 2.45 (t, J = 2.4 Hz, 1H), 2.75 (q, J = 14.8 Hz, 2H), 3.63 (s, 3H), 4.97 (s, 2H), 5.11 (dd, J = 2.4 Hz, 17.2 Hz, 1H), 5.13 (dd, J = 2.5 Hz, 17.1 Hz, 1H), 6.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.8, 32.0, 43.4, 43.7, 51.8, 71.9, 73.2, ~77 (one resonance is suspected to be under $CDCl_3$), 90.2, 98.8, 114.6, 128.4, 153.8, 157.4, 170.0; IR (CH_2Cl_2, NaCl, cm^{-1}) 3288, 2970, 2852, 2356, 2335, 1736, 1649, 1521, 1198, 1024; HRMS (EI) *m/z* calcd for $C_{15}H_{16}INO_4$ (M^+) 401.0124, found 401.0135; LRMS (EI) *m/z* 401 (M^+, 31), 372 (24), 345 (13), 327 (100), 245 (18), 206 (20), 162 (36), 77 (17).

(5-[3-{*tert*-Butyldimethylsilanyl}prop-2-ynyl]-1-ethyl-6-iodo-4-oxo-1,3,4,5-tetrahydro-furo[3,4-*c*]pyridin-1-yl) acetic acid methyl ester (16b). Following the above procedure, **15** (19 mg, 0.051 mmol) was alkylated with 3-*tert*-butyldimethylsilyl propargyl bromide (24 mg, 0.10 mmol) in the presence of NaH in mineral oil (60%, 2.3 mg, 0.056 mmol) and LiBr (8.9 mg, 0.10 mmol) in a mixture of DME (0.39 mL) and DMF (0.15 mL). The crude product was purified by flash chromatography (1:4 ethyl acetate/hexanes) to afford **16b** as a colorless oil (12 mg, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.82 (t, J = 7.4 Hz, 3H), 0.93 (s, 9H), 1.78 (dq, J = 7.4 Hz, 14.7 Hz, 1H), 1.92 (dq, J = 7.3 Hz, 14.7 Hz, 1H), 2.75 (q, J = 14.7 Hz, 2H), 3.62 (s, 3H), 4.98 (s, 2H), 5.12 (d, J = 17.3 Hz, 1H), 5.15 (d, J = 17.3 Hz, 1H), 6.76 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, 7.7, 16.6, 25.9, 26.0, 32.1, 43.8, 51.7, 72.0, 89.0, 90.2, 98.8, 99.3, 114.5, 128.2, 153.5, 157.3, 170.0; IR (CH_2Cl_2, NaCl, cm^{-1}) 2950, 2924, 2852, 2356, 2330, 1736, 1654, 1526, 1198, 1034; HRMS (EI) *m/z* calcd for $C_{21}H_{30}INO_4Si$ (M^+) 515.0989, found 515.0999; LRMS (EI) *m/z* 515 (M^+, 25), 458 (100), 441 (58), 420 (22), 384 (54), 356 (17), 258 (11), 162 (12), 96 (58), 73(57).

Methyl(3-ethyl-13-oxo-11,13-dihydro-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-*b*]quinolin-3-yl)acetate (17aa). Following general procedure A, iodopyridone **16a** (7.6 mg, 0.019 mmol) was reacted with phenyl isonitrile **13a** (1 M, 76 μL, 0.076 mmol) and hexamethylditin (11 μL, 0.028 mmol) in benzene (0.32 mL) to afford **17aa**, after purification, as a yellow solid (2.1 mg, 30%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, J = 7.3 Hz, 3H), 1.94 (dq, J = 7.2 Hz, 14.5 Hz, 1H), 2.05 (dq, J = 7.5 Hz, 14.7 Hz, 1H), 2.89 (d, J = 14.7 Hz, 1H), 2.90 (d, J = 14.8 Hz, 1H), 3.63 (s, 3H), 5.17 (d, J = 13.5 Hz, 1H), 5.18 (d, J = 13.4 Hz, 1H), 5.30 (s, 2H), 7.21 (s, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.83 (t, J = 7.1 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.39 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 7.9, 32.9, 44.4, 49.7, 51.8, 72.5, 90.8, 95.4, 127.8, 128.2, 128.3, 129.1, 129.2, 129.7, 130.6, 131.1, 147.2, 148.9, 152.9, 154.3, 157.1, 170.1; IR (CH_2Cl_2, NaCl, cm^{-1}) 2919, 2842, 2356, 2325, 1731, 1654, 1603, 1439, 1224, 1024; HRMS (EI) *m/z* calcd for $C_{22}H_{20}N_2O_4$ (M^+) 376 1423, found 376.1417; LRMS (EI) *m/z* 376 (M^+, 6), 347 (6), 302 (39), 261 (5), 137 (13), 97 (18), 81 (45), 69 (100).

Methyl(3-ethyl-8-fluoro-13-oxo-11,13-dihydro-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-*b*]quinolin-3-yl)acetate (17ba). Following general procedure A, iodopyridone **16a** (6.5 mg, 0.016 mmol) was reacted with *p*-fluorophenyl isonitrile **13b** (1 M, 65 μL, 0.065 mmol) and hexamethylditin (9.0 μL, 0.024 mmol) in benzene (0.27 mL) to afford **17ba**, after purification, as a yellow solid (3.5 mg, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 7.3 Hz, 3H), 1.94 (dq, J = 7.5 Hz, 14.6 Hz, 1H), 2.05 (dq, J = 7.8 Hz, 15.1 Hz, 1H), 2.89 (d, J = 14.7 Hz, 1H), 2.91 (d, J = 14.7 Hz, 1H), 3.63 (s, 3H), 5.17 (s, 2H), 5.30 (s, 2H), 7.17 (s, 1H), 7.54–7.64 (m, 2H), 8.21 (dd, J = 5.3 Hz, 9.2 Hz, 1H), 8.33 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.8, 32.8, 44.3, 49.5, 51.8, 72.4, 90.7, 95.2, 111.3 (d, J_{CF} = 20.0 Hz), 121.0, 128.8 (d, J_{CF} = 11.3 Hz), 129.1, 130.2 (d, J_{CF} = 46.3 Hz), 132.0 (d, J_{CF} = 7.5 Hz), 145.9, 146.8, 152.4, 154.3, 157.0, 160 (d, J_{CF} = 225 Hz, estimated), 170.1; IR (CH_2Cl_2, NaCl, cm^{-1}) 2909, 2847, 2351, 2340, 1721, 1654, 1593, 1501, 1449, 1234, 1024; HRMS (EI) *m/z* calcd for $C_{22}H_{19}FN_2O_4$ (M^+) 394.1329, found 394.1326; LRMS (EI) *m/z* 394 (M^+, 18), 365 (15), 337 (10), 320 (100), 293 (14), 171 (20), 105 (30), 83 (33), 69 (52).

Methyl{10-[*tert*-butyl(dimethyl)silyl]-3-ethyl-13-oxo-11,13-dihydro-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-*b*]quinolin-3-yl)}acetate (17ab). Following general procedure A, iodopyridone **16b** (8.3 mg, 0.016 mmol) was reacted with phenyl isonitrile **13a** (1 M, 64 μL, 0.064 mmol) and hexamethylditin (9.0 μL, 0.024 mmol) in benzene (0.27 mL) to afford **17ab**, after purification, as a yellow solid (5.6 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (s, 6H), 0.86 (t, J = 7.3 Hz, 3H), 1.01 (s, 9H), 1.94 (dq, J = 7.3 Hz, 14.6 Hz, 1H), 2.05 (dq, J = 7.3 Hz, 14.7 Hz, 1H), 2.89 (d, J = 14.7 Hz, 1H), 2.90 (d, J = 14.7 Hz, 1H), 3.63 (s, 3H), 5.16 (d, J = 13.4 Hz, 1H), 5.18 (d, J = 13.4 Hz, 1H), 5.32 (s, 2H), 7.19 (s, 1H), 7.61 (t, J = 7.3 Hz, 1H), 7.78 (t, J = 7.3 Hz, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 8.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −0.5, 7.7, 19.2, 27.1, 32.7, 44.3, 51.7, 52.0, 72.5, 90.6, 94.7, 126.7, 128.5, 129.5, 130.5, 132.7, 136.6, 143.0, 147.0, 148.0, 151.0, 154.1, 155.2, 156.8, 170.0; IR (CH_2Cl_2, NaCl, cm^{-1}) 2960, 2852, 2361, 2340, 1741, 1659, 1598, 1557, 1214, 1024; HRMS (EI) *m/z* calcd for $C_{28}H_{34}N_2O_4Si$ (M^+) 490.2288, found 490.2293; LRMS (EI) *m/z* 490 (M^+, 27), 461 (15), 434 (10), 416 (100), 359 (14), 331 (7), 73 (8).

Methyl{10-[*tert*-butyl(dimethyl)silyl]-3-ethyl-8-fluoro-13-oxo-11,13-dihydro-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-*b*]quinolin-3-yl)}acetate (17bb). Following general procedure A, iodopyridone **16b** (7.7 mg, 0.015 mmol) was reacted with *p*-fluorophenyl isonitrile **13b** (1 M, 60 μL, 0.060 mmol) and hexamethylditin (9.0 μL, 0.022 mmol) in benzene (0.25 mL) to afford **17bb**, after purification, as a yellow solid (5.0 mg, 66%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71 (s, 6H), 0.85 (t, J = 7.3 Hz, 3H), 1.01 (s, 9H), 1.94 (dq, J = 7.2 Hz, 14.4 Hz, 1H), 2.05 (dq, J = 7.5 Hz, 14.9 Hz, 1H), 2.89 (d, J = 14.7 Hz, 1H), 2.90 (d, J = 14.7 Hz, 1H), 3.63 (s, 3H), 5.16 (s, 2H), 5.31 (s, 2H), 7.16 (s, 1H), 7.56 (ddd, J = 2.7 Hz, 7.6 Hz, 10.1 Hz, 1H), 7.87 (dd, J = 2.7 Hz, 11.1 Hz, 1H), 8.20 (dd, J = 6.0 Hz, 9.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −0.7, 7.8, 19.2, 27.1, 32.8, 44.3, 51.8, 52.0, 72.5, 90.7, 94.8, 113.5 (d, J_{CF} = 24.0 Hz), 120.0 (d, J_{CF} = 25.0 Hz), 128.6, 133.5, 132.8, 137.5, 142.2, 145.1, 146.8, 150.7, 154.2, 156.8, 160 (d, J_{CF} = 225 Hz, estimated), 170.1; IR (CH_2Cl_2, NaCl, cm^{-1}) 2955, 2929, 2852, 2366, 2335, 1736, 1664, 1593, 1501, 1219; HRMS (EI) *m*/*z* calcd for $C_{28}H_{33}FN_2O_4Si$ (M^+) 508.2194, found 508.2199; LRMS (EI) *m*/*z* 508 (M^+, 22), 479 (14), 452 (10), 434 (100), 393 (13), 349 (6), 73 (5).

(*S*)-2-Hydroxy-2-(8-methyl-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl)butanoic acid methyl ester (19a). Triethylamine (0.17 mL, 1.2 mmol) was added to a solution of camptothecin **1** (27 mg, 0.078 mmol) in DMF (1.7 mL) under argon. Dry 10% palladium on activated carbon (5 mg) was carefully added to the reaction mixture and the dissolved oxygen was removed under vacuum. Then a balloon of hydrogen was mounted and the mixture was stirred vigorously for 24 h. The catalyst was removed by filtration of the reaction mixture through a pad of Celite. The filtrate was concentrated under reduced pressure to remove solvents and excess reagents. The crude product was purified by semipreparative HPLC using Symmetry C18 column under isocratic elution conditions (30:70 MeOH/H_2O + 0.1% HCOOH) to afford acid **18a** as a yellow solid (11 mg, 40%). This was used immediately in the subsequent reaction.

$TMSCHN_2$ (2 M solution in hexanes, 20 μL, 0.041 mmol) was added to a solution of **18a** (11 mg, 0.031 mmol) in a mixture of methanol (0.10 mL) and benzene (0.21 mL) at room temperature. After 30 min, the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (gradient elution 1:4 to 7:3 acetone/dichloromethane) to afford the ester **19a** as a pale yellow solid (8.6 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$, OH signal not located) δ 1.05 (t, J = 7.3 Hz, 3H), 2.31 (s, 3H), 2.37 (q, J = 7.5 Hz, 2H), 3.79 (s, 3H), 5.24 (s, 2H), 7.57 (s, 1H), 7.58 (t, J = 7.4 Hz, 1H), 7.78 (t, J = 8.4 Hz, 1H), 7.82 (d, J = 8.2 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.27 (s, 1H); HRMS (EI) *m*/*z* calcd for $C_{21}H_{20}N_2O_4$ (M^+) 364.1423, found 364.1406; LRMS (EI) *m*/*z* 364 (M^+, 67), 346 (19), 305 (100), 276 (65), 248 (35), 219 (45), 140 (13), 75 (15).

(*S*)-2-{12-[*tert*-Butyl(dimethyl)silyl]-2-hydroxy-8-methyl-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl}-2-hydroxybutanoic acid (18b). Triethylamine (98 μL, 0.70 mmol) was added to a solution of 7-*tert*-butyldimethylsilyl-10-hydroxy camptothecin (DB-67, 21 mg, 0.044 mmol) in MeOH (0.98 mL) under argon. Dry 10% palladium on activated carbon (4 mg) was carefully added to the reaction mixture and the dissolved oxygen was removed under vacuum. Then a balloon of hydrogen was mounted and the mixture was stirred vigorously for 14 h. The catalyst was removed by filtration of the reaction mixture through a pad of Celite. The filtrate was concentrated under reduced pressure to remove solvents and excess reagents. The crude product was purified by preparative TLC under isocratic elution conditions (95:5:5 dichloromethane/methanol/water) to afford **18b** as a yellow solid (17 mg, 83%): ^{1}H NMR (300 MHz, CD_3SOCD_3, two OH signals not located) δ 0.68 (s, 6H), 0.88 (t, J = 6.8 Hz, 3H), 0.98 (s, 9H), 1.88–2.09 (m, 2H), 2.24 (s, 3H), 5.10 (s, 2H), 7.33 (dd, J = 2.4 Hz, 9.0 Hz, 1H), 7.45 (s, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.99 (d, J = 9.1 Hz, 1H), 10.24 (br, 1H); ^{13}C NMR (75 MHz, CD_3SOCD_3) δ −1.1, 8.9, 13.9, 18.9, 27.1, 31.1, 52.1, 78.7, 99.1, 110.9, 121.8, 125.9, 131.3, 133.5, 137.0, 138.4, 140.6, 142.3, 148.9, 153.7, 155.9, 161.1, 175.4.

(*S*)-2-Hydroxy-2-[8-(hydroxymethyl)-9-oxo-9,11-dihydroindolizino[1,2-b]quinolin-7-yl]butanohydrazide (20a). Hydrazine monohydrate (45 μL, 0.93 mmol) was added to a suspension of camptothecin (54 mg, 0.15 mmol) in MeOH (0.62 mL) at room temperature. After stirring at the same temperature for 8 h, the reaction mixture was concentrated under reduced pressure to afford **20a** as a pale yellow solid (59 mg, 100%) which was sufficiently pure for further analysis: ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 0.85 (t, J = 7.0 Hz, 3H), 2.17 (d, J = 6.8 Hz, 2H), 4.29 (br s, 2H), 4.75 (dd, J = 5.7 Hz, 11.5 Hz, 1H), 4.77 (dd, J = 5.5 Hz, 11.6 Hz, 1H), 5.00 (t, J = 5.6Hz, 1H), 5.23 (s, 2H), 6.40 (br s, 1H), 7.46 (s, 1H), 7.69 (t, J = 7.4 Hz, 1H), 7.84 (t, J = 7.1 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.65 (s, 1H), 9.25 (br s, 1H); ^{13}C NMR (75 MHz, CD_3SOCD_3) δ 7.8, 31.8, 50.1, 55.3, 79.3, 99.3, 127.4, 127.7, 128.4, 128.8, 128.9, 129.7, 130.2, 131.3, 142.6, 147.9, 152.8, 152.9, 160.9, 171.7; HRMS (EI) *m*/*z* calcd for $C_{20}H_{16}N_2O_4$ ($M-N_2H_4$) 348.1110, found 348.1100; LRMS (EI) *m*/*z* 378 (M^+-2, 31), 348 (100), 319 (30), 289 (22), 248 (38), 219 (34), 140 (15).

(*S*)-2-[12-[*tert*-Butyl(dimethyl)silyl]-2-hydroxy-8-(hydroxymethyl)-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl]-2-hydroxybutanohydrazide (20b). Following the above procedure, 7-*tert*-butyldimethylsilyl-10-hydroxycamptothecin (DB-67) (24 mg, 0.050 mmol) was reacted with hydrazine monohydrate (24 μL, 0.50 mmol) in MeOH (0.20 mL) to afford **20b** as a yellow solid (25 mg, 99%) which was sufficiently pure for subsequent analysis: ^{1}H NMR (300 MHz, CD_3SOCD_3, four OH/NH signals not located) δ 0.64 (s, 6H), 0.84 (t, J = 6.8 Hz, 3H), 0.93 (s, 9H), 2.15 (d, J = 6.9 Hz, 1H), 4.27 (br s, 2H), 4.67 (d, J = 11.6 Hz, 1H), 4.81 (d, J = 11.7 Hz, 1H), 5.16 (s, 2H), 7.35–7.38 (m, 2H), 7.54 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 9.1 Hz, 1H), 9.21 (br, 1H); ^{13}C NMR (75 MHz, CD_3SOCD_3) δ −1.1, 7.9, 18.8, 27.1, 31.8, 48.6, 52.3, 79.3, 98.1, 110.8, 122.4, 127.5, 131.3, 133.9, 137.1, 138.2, 142.1, 143.1, 147.8, 153.2, 156.9, 160.7,

171.9; HRMS (EI) *m/z* calcd for $C_{26}H_{30}N_2O_5Si$ ($M^+-N_2H_4$) 478.1924, found 478.1906; LRMS (EI) *m/z* 478 ($M^+-N_2H_4$, 22), 434 (59), 421 (23), 377 (100), 320 (11), 291 (13), 235 (6), 73 (17).

(*S*)-2-[8-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl]-2-hydroxy-*N*-methoxy-*N*-methylbutanamide (22). Trimethylaluminum (2 M in heptane, 0.52 mL, 1.0 mmol) was added dropwise via a syringe over 3–4 min to a suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (0.10 g, 1.0 mmol) in CH_2Cl_2 (5.4 mL) at −10 °C accompanied by the evolution of gas. The resulting colorless solution was stirred at room temperature for 30 min and recooled to 0 °C. A suspension of camptothecin (0.12 g, 0.34 mmol) in CH_2Cl_2 (1.5 mL) was then added and the resulting clear brown solution was stirred at room temperature for 22 h. $NaHSO_4$ (1 M, 1.2 mL) was carefully added and the resulting mixture was extracted with CH_2Cl_2 (3× 10 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure to afford **21** as a yellow solid (96 mg, 68%). The crude product was used in the subsequent reaction immediately after the workup.

Alcohol **21** (95 mg, 0.23 mmol) was added to a solution of *tert*-butylchlorodimethylsilane (53 mg, 0.35 mmol) and imidazole (44 mg, 0.64 mmol) in DMF (0.19 mL). The reaction mixture was heated to 35 °C and stirred for 24 h. The reaction mixture was diluted with water (5 mL) and then extracted with ethyl acetate (3× 5 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford the Weinreb amide **22** as a pale yellow solid (0.11 g, 92%). The crude product was sufficiently pure for subsequent analysis. However, an analytical sample of **22** was prepared by purification of the crude product by flash column chromatography (step gradient elution 1:49, 1:19, 1:9 acetone/dichloromethane): ^1H NMR (300 MHz, $CDCl_3$) δ 0.19 (s, 3H), 0.22 (s, 3H), 0.96 (s, 9H), 0.97 (t, J = 7.3 Hz, 3H), 2.18 (dq, J = 7.4 Hz, 14.1 Hz, 1H), 2.44 (dq, J = 7.3 Hz, 13.9 Hz, 1H), 3.22 (s, 3H), 3.24 (s, 3H), 5.01 (d, J = 10.6 Hz, 1H), 5.02 (d, J = 10.7 Hz, 1H), 5.26 (d, J = 19.0 Hz, 1H), 5.30 (d, J = 19.0 Hz, 1H), 5.41 (br s, 1H), 7.37 (s, 1H), 7.65 (ddd, J = 1.1 Hz, 6.9 Hz, 8.1 Hz, 1H), 7.82 (ddd, J = 1.4 Hz, 6.9 Hz, 8.4 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.37 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.4, −5.2, 7.6, 18.5, 26.0, 31.4, 33.3, 50.1, 56.5, 59.9, 80.0, 99.4, 127.5, 127.7, 128.0, 128.1, 128.8, 129.6, 130.4, 130.9, 143.8, 148.8, 152.9, 154.4, 161.3, 172.9; IR (CH_2Cl_2, NaCl, cm^{-1}) 3370, 2929, 2847, 1659, 1603, 1465, 1398, 1250, 1055; HRMS (EI) *m/z* calcd for $C_{28}H_{37}N_3O_5Si$ (M^+) 523.2502, found 523.2477; LRMS (EI) *m/z* 523 (M^+, 18), 508 (28), 466 (100), 377 (98), 363 (28), 303 (60), 275 (15), 191 (7), 75(72).

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